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A COMMON INFUSION FLAGELLATE OCCURRING IN THE CAECAL CONTENTS OF THE CHICKEN

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During the past year a small flagellate has been regularly encountered in cultures from material containing eggs of *Heterakis papillosa,* a parasite of the common fowl, although this material was obtained from various sources. It has also been found in infusions contaminated with the feces of the rabbit. While this flagellate shows features somewhat characteristic of free-living organisms, its presence in the above cultures might be explained as well by contamination with fecal material as by the accidental introduction of a free-living species. In fact it has been found in large numbers in the caecal contents of young chickens which had been fed Heterakis material, so that it is evidently adapted to entozoic life. On the other hand it is found multiplying profusely in a variety of media outside the animal body. According to Alexeieff (1912), certain species of the genus Bodo are not only widely distributed as free-living organisms but may also occur as accidental parasites, retaining under such conditions the same characteristics as shown in their free state.

The flagellate under discussion is evidently very widely distributed. Similar organisms were found by Wight and Lucké (1920) in postmortem cultures and smears from various organs of soldiers who died from influenza during the 1919 epidemic. The description and figures furnished by these authors do not coincide in every respect with our findings, possibly owing to differences in fixation and method of staining. On account of the variation indicated in the arrangement of the organelles and the body-outline, identification from their text is difficult but it appears probable that they dealt with the species here reported. In the present study also, great variation of form was noted in air-dried films, although the contour of the living organism is quite uniform. In attempting to identify this flagellate it has been found that descriptions of similar organisms are so incomplete and confusing that it seems best to describe the one in question somewhat in detail.

The organism was studied in fresh and stained preparations made from cultures at room temperature on "amoeba agar" (agar 14, sodium chloride 6, water 900), which proved to be the most satisfactory culture medium. It multiplied at 37.5 C on this medium, but did not persist long in cultures kept at this temperature. It was also cultivated at room temperature in Locke-Ringer solution, but died in a few days unless vegetable or animal material was added to the solution, when it remained alive for a long time. No attempt was made to isolate it from the bacteria with which it grew. The structural details were studied in stained preparations, after killing the organism with osmic acid vapor and fixing it by the usual methods. The smears were stained by Giemsa's method and with Heidenhain's iron-hematoxylin, the latter giving the best results. A detailed description follows:

GENERAL MORPHOLOGY

The organism is colorless and transparent, more or less kidney-shaped and about twice as long as broad, with a variable length of from 5 to 12μ. In life the anterior flagellum is not readily visible; at first sight only the long, stout trailing flagellum is noticed. The former springs from the anterior extremity and the latter from a depression situated at the junction of the anterior and middle thirds of the body, dividing it into a smaller anterior and larger posterior portion. The surface from which the trailing flagellum emerges is called, for the sake of convenience, the ventral surface and is somewhat concave. The other, or dorsal surface, is sharply convex and regular. The anterior extremity is small and transparent, somewhat pointed and turned toward the ventral surface. This portion will be termed the "beak." It sometimes shows a clear space in the protoplasmic border between the points where the flagella emerge. The latter may be traced in their intraprotoplasmic course toward the dorsal surface, where they converge in the vicinity of a refractile body. The posterior portion of the organism is rounded and more voluminous than the anterior and it shows the nucleus situated near and slightly posterior to the ventral depression. The karyosome is sometimes readily distinguished as a large, refractile central mass. The posterior portion also contains numerous digestive vacuoles.

The ellipsoid or rounded nucleus is situated in the middle third of the organism, always nearer the ventral or concave surface and sometimes even slightly bulging from it. In well-differentiated preparations it is vesicular in type with a large, centrally-situated karyosome in the form of a very compact mass of structureless chromatin, and a thin limiting membrane with scanty peripheral chromatin. The space between the karyosome and the nuclear membrane is clear and shows no granules.

The cytoplasm in this species is a colorless, transparent and somewhat granular substance, containing numerous digestive vacuoles, which include food particles, mainly cocci and small bacilli. Some very refractile granules are seen in fresh preparations, situated near the nucleus, in the space between it and the contractile vacuole. These granules take the chromatin stains, but are constant neither in position nor number.

The contractile vacuole is situated in the anterior third of the body, near the nucleus. It probably possesses an acid reaction as indicated by sensitized Neutral Red added to the culture. A very small, clear space was found in many of the living and fixed organisms, situated in the protoplasmic border of the "beak," just between the two points from which the flagella emerge (Fig. 6). Another vacuolar structure, possibly of the nature of a kinetonucleus, will be described later.

FLAGELLAR APPARATUS

The organism possesses two unequal flagella which arise separately from two indistinct granules which lie near a dorsally situated coarse mass of chromatin. Thence they cross the anterior portion of the organism to its ventral surface from which they emerge at some distance from one another. The more anterior flagellum, or tractellum, emerges from the anterior extremity; it is slender, short and never exceeds the length of the body. The trailing flagellum, or gubernaculum, is from two and a half to three times the length of the body and is considerably thicker than the anterior flagellum, especially at its base, where it is also quite rigid. It emerges from the ventral surface and is directed posteriorly.

At the origin of the flagella is a chromatin mass which lies against a clear, vacuolar space surrounded by a definite line, or ring, which takes the chromatin stain by both the Giemsa and Heidenhain methods; with the former it appears as a dull, pinkish mass, which often stains poorly, surrounded by a definite red ring; with the Heidenhain stain it shows either a lightly-stained substance, or merely a ring which is definitely darker than the contour of the ordinary vacuoles. This ring-like structure, which lies against the dorsal side (sometimes bulging from it as does the nucleus at the ventral side) may possibly represent the membrane of a kinetonucleus having a chromatin mass at its anterior pole. This interpretation must, however, be considered tentative for the present, as it is difficult to determine whether this ring is the demarcation line of the contractile vacuole, or a separate structure, for the organism suffers more or less change of form in stained preparations. Even with osmic acid fixation which gives good results in this particular case, the contractile vacuole varies from a median to a dorsal position. The peripheral position of the ring is suggestive of a contractile vacuole, as the latter (according to Minchin) is always an ectoplasmic organelle. Since the structure in question was never seen to contract, and because of its staining properties, its relation to the flagellar apparatus and its behavior during the process of division, there is much in favor of considering it as a true kinetonucleus. In the living flagellate, however, the contractile vacuole is always found in the anterior third, near the nucleus and very close to a bright granule from which the flagella seem to arise.

Hartmann and Chagas (1910) state that Bodo (Ehrenberg) has only one nucleus, in which the karyosome is connected by a rhizoplast with the basal granules from which the flagella arise and they consider the possession of a kinetonucleus and a special flagellar apparatus as characteristic of Prowazekia. On the other hand, Alexeieff (1912) states that the possession of a kinetonucleus is constant in Bodo.

MOVEMENTS

Under normal conditions when the organism is swimming free, it is impossible to follow it in its rapid motion through the microscopic field; but when the preparation begins to dry out, or when a viscid substance such as saliva is added to the culture, its movements can be followed and the flagella and their action readily studied.

The anterior flagellus or tractellum sweeps with broad undulations always toward the ventral side, moving the body by traction. The trailing flagellum or gubernaculum, which is rather stiff and thick at its base, extends posteriorly from the depression in the ventral surface in a slight curve, directed obliquely over its lateral surface from the antero-ventral to the postero-dorsal side (Figs. 1, 2). When the organism is at rest, attached by its trailing flagellum, the tractellum lies against the body, directed backward and parallel with the gubernaculum and at some distance from it. The latter, after leaving the immediate vicinity of the body, becomes gradually attenuated and exhibits broad undulations when the flagellate is in motion. At first glance the organism seems to progress in a spiral fashion, after the manner of the Rotatoria; but after careful examination it is seen that the rotation is incomplete and reversing in character. The combined action of both flagella gives to the flagellate a peculiar, semi-rotatory motion on its longitudinal axis combined with a lateral swaying about a point situated at the anterior extremity. Similar movements were described by Moroff (1904) in the case of *Bodo ovatus,* but the action of its anterior flagellum is quite different from that observed in our flagellate.

In some instances organisms were seen swimming slowly around a bacterial colony, with the dorsal surface toward the center of the circle described by their movements. Under these circumstances the anterior flagellum may be folded back against the surface of the body and is seen with difficulty. This peculiar motion seems to be due to the weak undulation of the tractellum together with a lack of control by the gubernaculum and is evidently abnormal, for organisms which showed this phenomenon died shortly afterward. When the flagellate is swimming through the field its trailing flagellum occasionally becomes attached to the glass, to a bacterial colony or to some solid particle, thus anchoring it in position. It endeavors to free itself by means of violent springing or jerking movements and then lies quiescent; but the slightest disturbance causes renewed contractions, which are sometimes so rapid as to compare with vibrations. The organism jerks and pulls at the mass at short intervals until, at last, it may succeed in freeing its sticky appendage and continues on its way. In some cases it may swim slowly, carrying with it adhering material, but often fails to free its attached extremity, remains a prisoner, and dies. This is apparently due to the adhesive properties of the trailing flagellum under temporary, artificial conditions and cannot be considered as a special mode of life, as Kent (1882) has claimed for *Diplomastix* (*Bodo*) *saltans* (Ehrenberg).

INGESTION OF FOOD

No definite mouth or cytostome is found; food is taken in through the depression on the ventral surface of the organism, this process being aided by the movements of the tractellum. The process was studied in both fresh and fixed preparations from cultures into which carmin or Higgins' waterproof drawing ink had been introduced. It is rather remarkable that the ingested particles have a tendency to arrange themselves at first around the nucleus (Figs. 3, 4, 5), this localization being noted in many living and fixed organisms studied soon after they had been given carmin or ink. These particles later become scattered throughout the cytoplasm, being enclosed in vacuoles and later often accumulate in a mass near the posterior extremity. On one occasion, an organism expelled all the ingested ink and became clear, but the point at which it was ejected was not evident.

MULTIPLICATION

This species multiplies by binary division (Figs. 13-17). The earliest stages noted showed three flagella and the kinetonucleus in the process of division. Later stages of division were seen in organisms that showed four flagella arising from two kinetonuclei (red rings with Giemsa's stain) or from granules very close to them. The kinetonucleus must divide very rapidly, for we could find no intermediate stages. When this body has already divided, the true nucleus is still single or in process of division. After the division of the kinetonucleus the nucleus becomes elongated and shows at its center indefinite masses of chromatin (Fig. 14). Pale, diffusely stained masses next appear at its poles, the karyosomic chromatin becomes arranged at the center and, when the nucleus is stretched out, this mass of chromatin assumes the appearance of two cones with their apices in contact and having at their bases the two rather clear unstained masses (Fig 15).

At the completion of nuclear division, a faint line is seen uniting the two newly formed nuclei (Fig. 16). Dividing organisms seem larger and more or less rounded. The fission of the cytoplasm begins at the flagellar extremity and progresses toward the posterior end, splitting the organism longitudinally (Fig. 17).

Small, rounded forms without flagella occur in old cultures, which may be interpreted as resting-forms, or "cysts" (Fig. 18).

CLASSIFICATION

It is evident that this flagellate belongs to the sub-order Monozoa, family Bodonidae and, according to Alexeieff's classification to the genus Bodo, which is defined by him as follows:

> "Body ovate, form variable for different species, constant for the same species. Two flagella, one anterior, one recurrent, generally larger, arise from a small granule situated in front of a voluminous chromophile body (kinetonucleus). Main nucleus with large karyosome, contractile vacuole single, toward the anterior extremity, near kinetonucleus. Nutrition by englobing solid parcles. They are free living, ubiquitous, sometimes presenting cases of accidental parasitism."

Hartmann and Chagas (1910) on the contrary consider the possesion of a kinetonucleus in organisms of this family, as a characteristic of the genus Prowazekia. They gave for the genus Bodo Ehrg. a flagellar apparatus consisting of two basal granules from which the flagella arise; these granules are connected with the karyosome by means of a rhizoplast.

These two conflicting statements have been a matter of lengthy discussion that will not be entered into this paper. It appears preferable to classify the organism in question in the genus *Bodo* Ehrenberg until the structure described tentatively as a kinetonucleus is demonstrated as such; and also until the absence of kinetonucleus is established for the genus Bodo and its presence accepted as diagnostic of the genus Prowazekia.

The nuclear character of kinetonuclei in general is questioned by Janecki and Kofoid who prefers the term parabasal for structures of this type. Considering the variations of such structures even within a given species, the question may be raised as to their importance as generic characters.

References to organisms similar to the one here described, are found in the literature and it may be well to consider them from a comparative point of view.

Pleuromonas jaculans Perty (1852). This organism is closely related to ours if it is not identical. The only difference found is with respect to the ingestion of food, which in Pleuromonas is, according to Perty, by means of a dorsal vacuole. In our flagellate food particles are taken in through the ventral depression at the base of the gubernaculum. Kent makes the following statement concerning this species: "It is further by no means improbable that the type *Pleuromonas* *jaculans,* upon which the genus has been founded, is identical with *Heteronema* (*Bodo*) *saltans* of Ehrenberg, and which in addition to exhibing similar leaping movements, appears under insufficient magnification to possess a single flagellum only." Thus while Perty's original description gives to Pleuromonas one single flagellum it probably possesses two as claimed by Kent (1880) and also later on by Conn (1905).

Anisonema grande Ehrenberg sp. differs from the species here described only in possessing "an oral aperture adjacent to the origin of the posterior flagellum and communicating with a short tubular pharyngeal tract," Kent (1882).

Bodo ovatus Moroff (1904). This flagellate differs from ours in that the nucleus is centrally situated and the flagella are totally different as regards position and length.

Diplomastix saltans Ehrenberg sp. first described by Ehrenberg as *Bodo saltans,* differs in possession of a bilabiated or notched depression in its anterior extremity, which is considered as a cystostome. Kent's description gives its dimensions as 15.6 by 28 μ, which are about twice those given by Conn for *Pleuromonas jaculans.* Its movements, as described by him, agree in the main with those of the species discussed in this paper, but its "sendentary habits" are notably absent in the latter.

The flagellate under consideration agrees most closely with the description of *Pleuromonas jaculans* Perty, as corrected by Kent and by Conn. While it appears probable that this is the species with which we are dealing, we cannot positively identify it as such on account of disagreement with respect to the manner of ingesting food. It is possible that Perty may have been in error concerning this question, but this assumption is unwarranted without a survey of all available related flagellates. The descriptions at hand suggest that various generic and specific names have been applied to single species and many descriptions are not only incomplete but probably also incorrect in certain respects. The confusion is such that any further classification of the flagellate under discussion will at present not be attempted.

The occurrence of this species in the caeca of chickens that had been fed with material containing it in large numbers indicates a condition of accidental parasitism, for it appears to be widely distributed and is found multiplying profusely outside of the animal body in a variety of media and at low temperatures.

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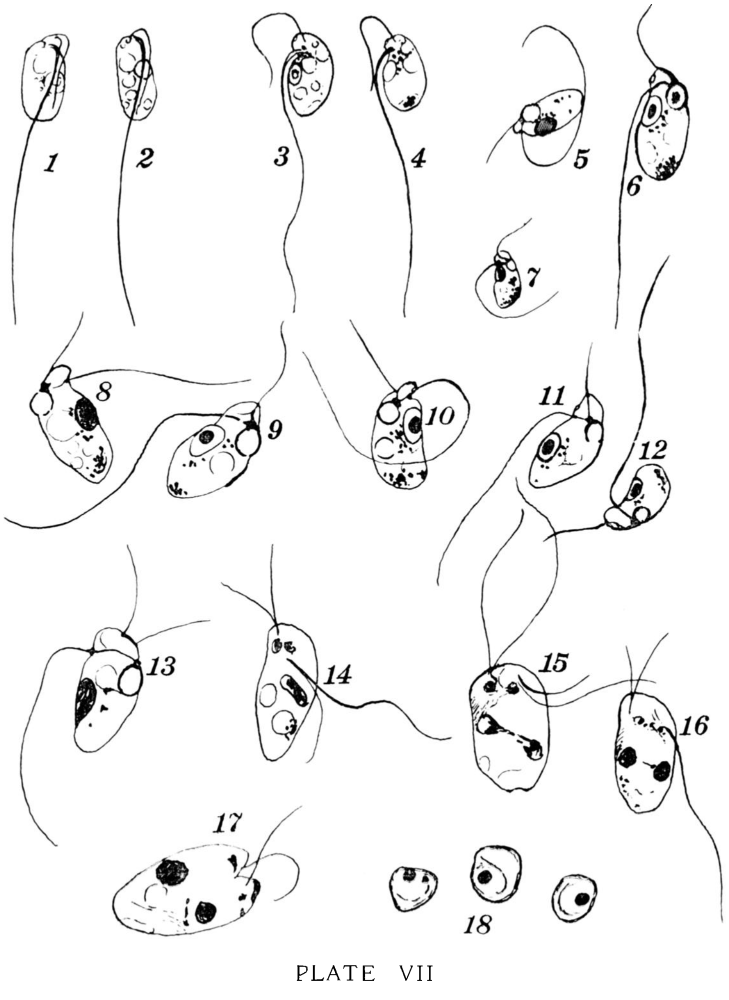

PLATE VII

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EXPLANATION OF PLATE VII

Figs. 1, 2.—Fresh preparation. Organisms attached by gubernaculum.

Figs. 3, 4.—Fresh preparation, showing successive positions of flagella during locomotion. Ink granules at base of gubernaculum.

Fig. 5.—Distribution of granules around the nucleus (Heidenhain's iron hematoxylin stain).

Fig. 6.—Showing small vacuole at "beak" (Heidenhain's stain).

Fig. 7.—Very small organism, minimum size (Heidenhain's stain).

Figs. 8-12.—Showing relation of flagella to kinetonucleus (Heidenhain's stain).

Fig. 13.—Dividing form with three flagella (Heidenhain's stain).

Fig. 14.—Main nucleus begins to divide, when kinetonucleus is already divided in two red masses (Giemsa's stain).

Fig. 15.—Division of main nucleus, with formation of polar masses united by karyosomic chromatin (Heidenhain's hematoxylin).

Fig. 16.—Advanced stage of division of main nucleus (Heidenhain's stain).

Fig. 17.—Binucleated organism splitting into two (Heidenhain's stain).

Fig. 18.—Small aflagellar forms, suggesting resistant forms or cysts (Heidenhain's stain).